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Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: METHODS FOR THE PRODUCTION OF TCR GAMMA DELTA T CELLS

(57) Abstract

The method for obtaining and expanding TcR$\gamma\delta^+$ T cells in culture is described. The method involves: 1) culturing cells from a sample containing TcR$\gamma\delta^+$ T cells or precursors thereof in a first culture medium comprising a T cell mitogen and at least two cytokines and 2) culturing the cells obtained in step 1) in a second culture medium comprising at least two cytokines. Preferably, the method comprises 1) culturing the cells in a first culture medium comprising (a) a T cell mitogen, (b) interleukin–2 and (c) interleukin–4; and 2) culturing the cells obtained in step 1) in a second culture medium comprising (i) interleukin–2 and (ii) interleukin–4 to obtain TcR$\gamma\delta^+$ T cells. The TcR$\gamma\delta^+$ T cells obtained by the method can be used in a variety of experimental, therapeutic and commercial applications.

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INTERNATIONAL SEARCH REPORT

Int. tional Application No
PCT/CA 99/01024

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N5/06 C12N5/08 A61P37/02 A61P35/00 A61P31/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98 33891 A (HEMOSOL INC) 6 August 1998 (1998-08-06) cited in the application page 9, line 3 - line 6 page 9, line 22 - line 29 page 13, line 11 - line 22 page 13, line 28 -page 15, line 25 page 16, line 16 - line 17 example 1; table 7 page 31, line 19 - line 28 claims 1-56 --- -/--	1,34-37, 41-50

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
15 February 2000	09/03/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Fernandez y Branas, F

1

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Int ional Application No
PCT/CA 99/01024

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	BARCENA A ET AL: "A role for interleukin 4 in the differentiation of mature T cell receptor gamma / delta + cells from human intrathymic T cell precursors." JOURNAL OF EXPERIMENTAL MEDICINE, (1990 AUG 1) 172 (2) 439-46. , XP000876697 page 441, column 2, paragraphs 1,3; table 1 ---	34-40
X	WO 98 19167 A (SPIES THOMAS ;SPIES VERONIKA (US); HUTCHINSON FRED CANCER RES (US)) 7 May 1998 (1998-05-07) claims 26-52 claims 74-79 ---	34-42, 44,47,49
X	US 5 639 653 A (BLOOM BARRY R ET AL) 17 June 1997 (1997-06-17) the whole document ---	34-37, 41-50
X,P	WO 99 46365 A (LOPEZ RICHARD ;NEGRIN ROBERT (US); UNIV EMORY (US); WALLER EDMUND) 16 September 1999 (1999-09-16) the whole document -----	35-37, 41-44, 47-49

1

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA 99/01024

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Remark: Although claims 47-50 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Ir tional Application No
PCT/CA 99/01024

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9833891 A	06-08-1998	AU 5744798 A EP 0966523 A	25-08-1998 29-12-1999
WO 9819167 A	07-05-1998	EP 0937258 A	25-08-1999
US 5639653 A	17-06-1997	US 5902793 A	11-05-1999
WO 9946365 A	16-09-1999	AU 2999299 A	27-09-1999

WE CLAIM:

1. A method for expanding TcR$\gamma\delta^+$ T cells in a starting sample comprising:

(1) culturing cells in the starting sample in a first culture medium comprising a T cell mitogen and at least two cytokines; and

(2) culturing the cells obtained in step (1) in a second culture medium comprising at least two cytokines to expand TcR$\gamma\delta^+$ T cells.

2. A method according to claim 1 wherein the first culture medium comprises (a) a T cell mitogen, (b) interleukin-2 and (c) interleukin-4; and the second culture medium comprises (i) interleukin-2 and (ii) interleukin-4.

3. A method according to claim 1 wherein the first culture medium comprises a leukocyte conditioned medium and the second culture medium comprises (i) interleukin-2 and (ii) interleukin-4.

4. A method according to claim 3 wherein the leukocyte conditioned medium is XLCM.

5. A method according to any one of claims 1 to 4 wherein the first and second culture media contain serum or plasma.

6. A method according to any one of claims 1 to 5 wherein prior to step (1) the cells in the starting sample are enriched for T cells.

7. A method according to any one of claims 1 to 6 wherein prior to step (1) the cells in the starting sample are enriched for CD4$^+$ cells.

8. A method according to any one of claims 1 to 7 wherein prior to step (1) the cells in the starting sample are depleted of $CD14^+$, $CD16^+$, $CD19^+$, $CD56^+$ and glycophorin A^+ cells.

9. A method according to any one of claims 1 to 8 wherein prior to step (1) the cells in the starting sample are depleted of $TcR\alpha\beta^+$ T cells.

10. A method according to any one of claims 1 to 9 wherein prior to step (1) the cells in the starting sample are depleted of non-$TcR\gamma\delta^+$ T cells.

11. A method according to any one of claims 1 to 10 wherein the starting sample is selected from peripheral blood, bone marrow, lymphoid tissue, epithelia, thymus, liver, spleen, cancerous tissue, infected tissue, lymph node tissue or fractions thereof.

12. A method according to claim 11 wherein the starting sample is human peripheral blood or a fraction thereof.

13. A method according to any one of claims 1 to 12 wherein the starting sample is low density mononuclear cells.

14. A method according to any one of claims 2 to 13 wherein in the first culture medium the T cell mitogen is present in an amount from about 0.01 to about 100 µg/ml; the IL-2 is present in an amount from about 0.1 to about 1000 ng/ml and the IL-4 is present in an amount from about 0.1 to about 1000 ng/ml.

15. A method according to any one of claims 2 to 13 wherein in the first culture medium the T cell mitogen is present in an amount from about 0.1 to about 50 µg/ml; the IL-2 is present in an amount from about 1

to about 100 ng/ml and the IL-4 is present in an amount from about 1 to about 100 ng/ml.

16. A method according to any one of claims 2 to 13 wherein in the first culture medium the T cell mitogen is present in an amount from about 0.5 to about 10 µg/ml; the IL-2 is present in an amount from about 2 to about 50 ng/ml and the IL-4 is present in an amount from about 2 to about 50 ng/ml.

17. A method according to any one of claims 1 to 16 wherein the first culture medium comprises 1µg/mL of a T cell mitogen; 10 ng/mL IL-2 and 10 ng/mL IL-4.

18. A method according to any one of claims 14 to 17 wherein the T cell mitogen is concanavalin A.

19. A method according to claim 5 wherein the serum or plasma is present in an amount from about 1 to about 25% by volume.

20. A method according to claim 5 wherein the serum or plasma is present in an amount from about 2 to about 20% by volume.

21. A method according to claim 5 wherein the serum or plasma is present in an amount from about 2.5 to about 10% by volume.

22. A method according to claim 5 wherein the serum or plasma is present in an amount of about 5% by volume.

23. A method according to any one of claims 2 to 22 wherein in the second culture medium the IL-2 is present in an amount from about 0.1 to about 1000 ng/ml and the IL-4 is present in an amount from about 0.1 to about 1000 ng/ml.

24. A method according to any one of claims 2 to 22 wherein in the second culture medium the IL-2 is present in an amount from about 1 to about 100 ng/ml and the IL-4 is present in an amount from about 1 to about 100 ng/ml.

25. A method according to any one of claims 2 to 22 wherein in the second culture medium the IL-2 is present in an amount from about 2 to about 50 ng/ml and the IL-4 is present in an amount from about 2 to about 50 ng/ml.

26. A method according to any one of claims 1 to 25 wherein the second culture medium comprises 10 ng/mL IL-2 and 10 ng/mL IL-4.

27. A method according to claim 4 wherein the XLCM is present in an amount from about 1 to about 25%.

28. A method according to claim 4 wherein the XLCM is present in an amount from about 2 to about 20%.

29. A method according to claim 4 wherein the XLCM is present in an amount from about 2.5 to about 10%.

30. A method according to claim 4 wherein the XLCM is present in an amount from about 5%.

31. A method for obtaining TcR$\gamma\delta^+$ T cells from a sample from a patient with chronic myelogenous leukemia comprising:

(1) obtaining low density mononuclear cells (LDMNC) from the sample;

(2) depleting the cells obtained in step (1) of $CD33^+$ cells;

(3) culturing the cells obtained in step (2) in a first culture medium comprising (a) a T cell mitogen, (b) interleukin-2 and (c) interleukin-4; and

(4) culturing the cells obtained in step (3) in a second culture medium comprising (i) interleukin-2 and (ii) interleukin-4 to expand TcR$\gamma\delta^+$ T cells.

32. A method according to claim 31 wherein step (2) additionally comprises depleting the cells of $CD14^+$, $CD16^+$, $CD19^+$, $CD56^+$ and glycophorin A^+ cells.

33. A method according to claim 31 wherein step (2) additionally comprises depleting the cells of TcR$\alpha\beta^+$ T cells.

34. A cell preparation enriched in TcR$\gamma\delta^+$ T cells prepared according to the method of any one of claims 1 to 33.

35. A cell preparation enriched in TcR$\gamma\delta^+$ T cells wherein greater than 70% of the total cells are TcR$\gamma\delta^+$ T cells.

36. A cell preparation according to claims 34 or 35 wherein greater than 80% of the total cells are TcR$\gamma\delta^+$ T cells.

37. A cell preparation according to claims 34, 35 or 36 wherein greater than 90% of the total cells are TcR$\gamma\delta^+$ T cells.

38. A cell preparation according to any one of claims 34 to 37 which comprises V$\delta1^+$ and V$\delta2^+$ TcR$\gamma\delta^+$ T cells.

39. A cell preparation according to claim 38 which comprises about 50-90% $V\delta1^+$ and about 10-50% $V\delta2^+$ $TcR\gamma\delta^+$ T cells of the total $TcR\gamma\delta^+$ T cells in the preparation.

40. A cell preparation according to claim 38 which comprises about 70% $V\delta1^+$ and about 30% $V\delta2^+$ $TcR\gamma\delta^+$ T cells of the total $TcR\gamma\delta^+$ T cells in the preparation.

41. A cell preparation according to any one of claims 34 to 40 that is substantially free of a T cell mitogen.

42. A use of a cell preparation according to any one of claims 34 to 41 to prepare a medicament to modulate an immune response.

43. A use of a cell preparation according to any one of claims 34 to 41 to prepare a medicament to treat an infection.

44. A use of a cell preparation according to any one of claims 34 to 41 to prepare a medicament to treat cancer.

45. A use of a cell preparation according to any one of claims 34 to 41 to prepare a medicament to treat chronic myelogenous leukemia.

46. A use of a cell preparation according to any one of claims 34 to 41 to prepare a vaccine.

47. A method of modulating an immune response comprising administering an effective amount of $TcR\gamma\delta^+$ T cells obtained according to the method of any one of claims 1 to 33 or obtained from a cell preparation according to any one of claims 34 to 41 to an animal in need thereof.

48. A method for treating an infection comprising administering an effective amount of TcR$\gamma\delta^+$ T cells obtained according to the method of any one of claims 1 to 33 or obtained from a cell preparation according to any one of claims 34 to 41 to an animal in need thereof.

49. A method for treating cancer comprising administering an effective amount of TcR$\gamma\delta^+$ T cells obtained according to the method of any one of claims 1 to 33 or obtained from a cell preparation according to any one of claims 34 to 41 to an animal in need thereof.

50. A method for treating chronic myelogenous leukemia comprising administering an effective amount of TcR$\gamma\delta^+$ T cells obtained according to the method of any one of claims 1 to 33 or obtained from a cell preparation according to any one of claims 34 to 41 to an animal in need thereof.

09/807987
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PATENT COOPERATION TREATY

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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

14

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
6704-3		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/CA99/01024	04/11/1999	04/11/1998
International Patent Classification (IPC) or national classification and IPC C12N5/06		
Applicant HEMOSOL INC. et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 7 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 1 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
30/05/2000	16.02.2001
Name and mailing address of the international preliminary examining authority: European Patent Office - P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Tx: 31 651 epo nl Fax: +31 70 340 - 3016	Authorized officer Fernandez y Branas,F Telephone No. +31 70 340 2774

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/CA99/01024

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:
Description, pages:

1-32	as originally filed				

Claims, No.:

1-47	as received on	02/11/2000	with letter of	02/11/2000	

Drawings, sheets:

1/12-12/12	as originally filed				

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   - ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
   - ☐ the language of publication of the international application (under Rule 48.3(b)).
   - ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   - ☐ contained in the international application in written form.
   - ☐ filed together with the international application in computer readable form.
   - ☐ furnished subsequently to this Authority in written form.
   - ☐ furnished subsequently to this Authority in computer readable form.
   - ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
   - ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   - ☐ the description, pages:
   - ☐ the claims, Nos.:

☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

   ☐ the entire international application.

   ☒ claims Nos. 44-47 with respect to Industrial Applicability.

because:

   ☒ the said international application, or the said claims Nos. 44-47 relate to the following subject matter which does not require an international preliminary examination (*specify*):
   see separate sheet

   ☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

   ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

   ☐ no international search report has been established for the said claims Nos. .

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

   ☐ the written form has not been furnished or does not comply with the standard.

   ☐ the computer readable form has not been furnished or does not comply with the standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

   Novelty (N) Yes: Claims 1-31 42 47

	No:	Claims	32-41 43-46
Inventive step (IS)	Yes:	Claims	1-31
	No:	Claims	32-47
Industrial applicability (IA)	Yes:	Claims	1-43
	No:	Claims	

2. Citations and explanations
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

Claims 44-47 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(i) PCT).

Re Item V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

D1.....WO-A-9833891 (HEMOSOL INC)
D2.....Journal of Experimental Medicine, 172, 1990, pages 439-446
D3.....WO-A-9819167 (FRED HUTCHINSON CANCER RESEARCH CENTER INC.)

For the assessment of the present claims 39-47 on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

D1 discloses the culturing and expansion of lymphocyte populations including TcRγδ, and the use of such cells in the treatment of leukaemia, cancer and infections. The methods disclosed in D1 use a Conditioned Medium (CM) obtained by stimulating peripheral blood mononuclear cells, low density mononuclear cells, buffy coats...etc with mitogens. The composition of the CM is said to contain multiple cytokines. Il-2 and Il-4 are also included in the composition of said CM. By FACS the proportion of TcRγδ+ cells is as high as 64%, see passages cited in the search report.

Claims for products defined in terms of a process of manufacture should fulfil as such the requirements for patentability, i.e. inter alia that they are new and inventive. Therefore, a product is not rendered novel and inventive merely by the fact that it is produced by means of a new and inventive process. Novelty and inventive step of the product should derive from the technical characteristics of the product self.

Consequently, and in view of D1, the subject matter of claims 32, 39-41 and 43-46 lacks novelty in the sense of Article 33(2) PCT.

In addition, the fact that in example 8 of D1 the cells are separated by FACS analysis means that the TcRγδ+ cells are separated via the cell sorter and thus that populations of almost pure TcRγδ+ cells are described in D1. Thus, the subject matter of claims 33-35 also lacks novelty in the sense of Article 33(2) PCT.

D2 discloses the expansion of TcRγδ+ cells departing from thymocyte precursors in presence of IL-4. It is mentioned that 85% of the $CD3^+$ T cells generated in presence of IL-4 expressed the TcRγδ+, 46% of which were TcRγ/δ^1, see page 441, right column first paragraph and figure 4.

In view of D2, the subject matter of claims 32-35 lacks novelty according to Article 33(2) PCT.

Pure populations of TcR$\gamma\delta^1$ cells are described in D3, as well as their pharmaceutical use e.g. against cancer, see passages cited in the search report. Thus, in view of D3, the subject matter of claims 32-39, 41, 44 and 46 lacks novelty according to Article 33(2) PCT.

The subject matter of claims 42 and 47 derives in an obvious manner from D1, as the uses of TcR$\gamma\delta$+ cells to treat leukemia and cancer are described in this document. Therefore the subject matter of claims 42 and 47 lacks inventive step according to Article 33(3) PCT.

The subject matter of claims 1-31 appears to be new in the sense of Article 33(2) PCT. D1 does not disclose the (sub) culturing methods of claim 1. The conditioned medium of D1 contains IL-4 only in minute quantities almost beyond detection, see page 18, table 1 of D1; the culture in this medium and plasma from umbilical cord blood (see page 20, lines 24-25) mostly expands TcR$\alpha\beta$ cells, although at the end of the culture TcR$\gamma\delta$+ also develop, see example 8 of D1. In example 5 of the present application it is stated that the subculturing in a medium containing defined cytokines was done with the purpose of eliminating the conditioned medium and the residual T-cell mitogens. The result, as shown in example 5, is that the expansion of T cells was maintained with the subset of TcR$\gamma\delta$+ preferentially expanded. Thus, the IPEA is of the opinion that when reading claims 1-2 the skilled person understands that a) the cells should be cultured in two different culture media (1) and (2), the second medium (2) being deprived of T-cell mitogen.

Said claims 1-31 appear to involve an inventive step in the sense of Article 33(3) PCT, as there is no suggestion in the prior art towards the methods claimed, bearing in mind that the IL-4 would have been disregarded by the skilled person upon reading D1, table 1, and taking into account the technical effects shown in figures 3 and 5 concerning the

expansion of TcRγδ+ cells following the said methods..

Re Item VIII

Certain observations on the international application

The term "XLCM" (claim 2 and 25-28) is unclear.

The term "about" (claims 12-14, 17-28) is unclear

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Assistant Commissioner for Patents
United States Patent and Trademark Office
Box PCT
Washington, D.C.20231
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 22 June 2000 (22.06.00)	
International application No. PCT/CA99/01024	**Applicant's or agent's file reference** 6704-3
International filing date (day/month/year) 04 November 1999 (04.11.99)	**Priority date** (day/month/year) 04 November 1998 (04.11.98)
Applicant BELL, David, Nicholson et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

30 May 2000 (30.05.00)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer F. Baechler
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